

Figure S1

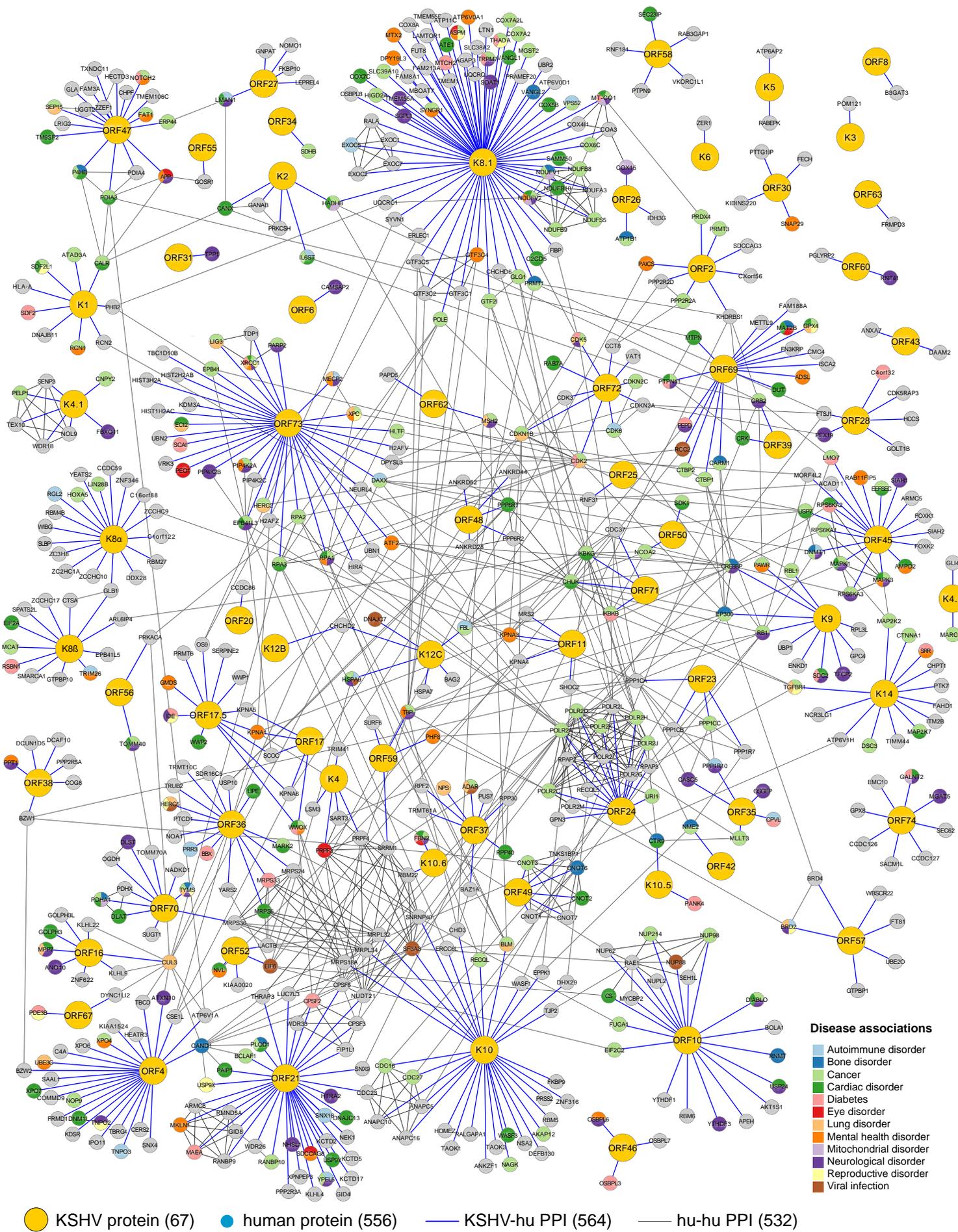


Figure S2

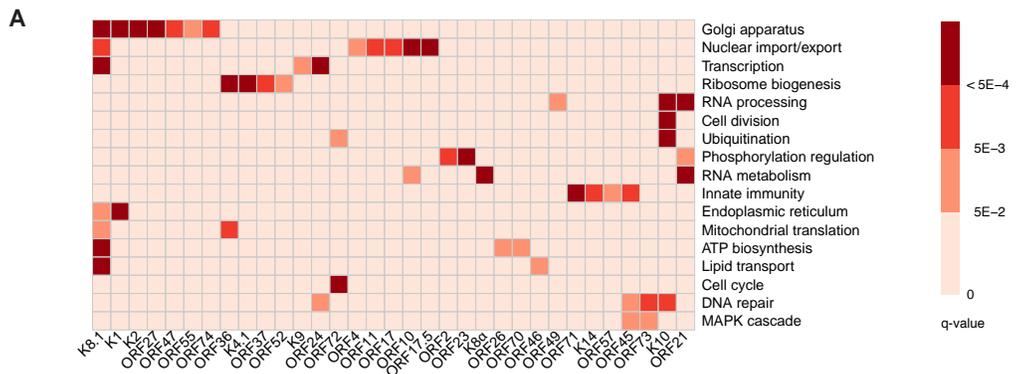


Figure S3

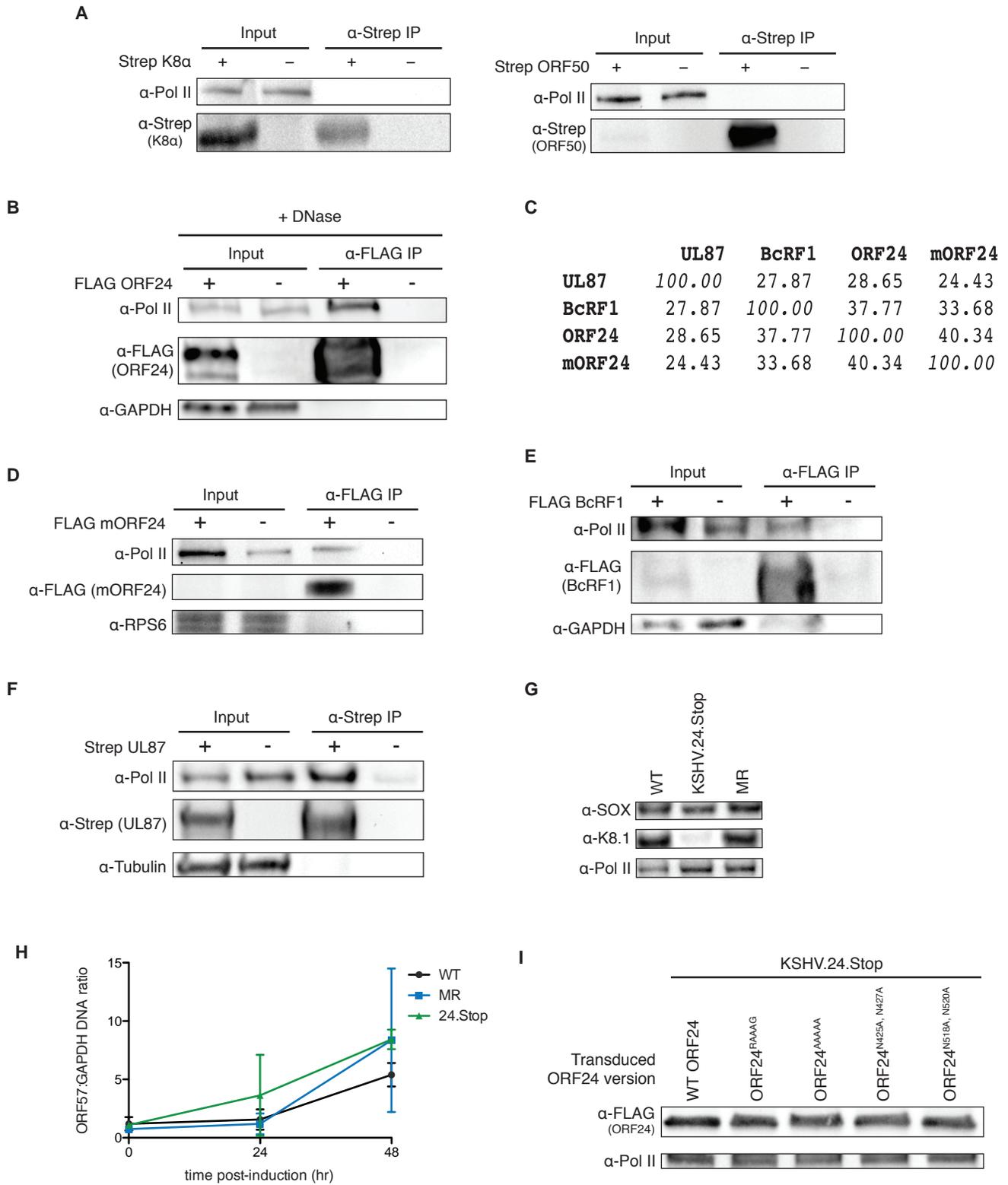


Figure S4

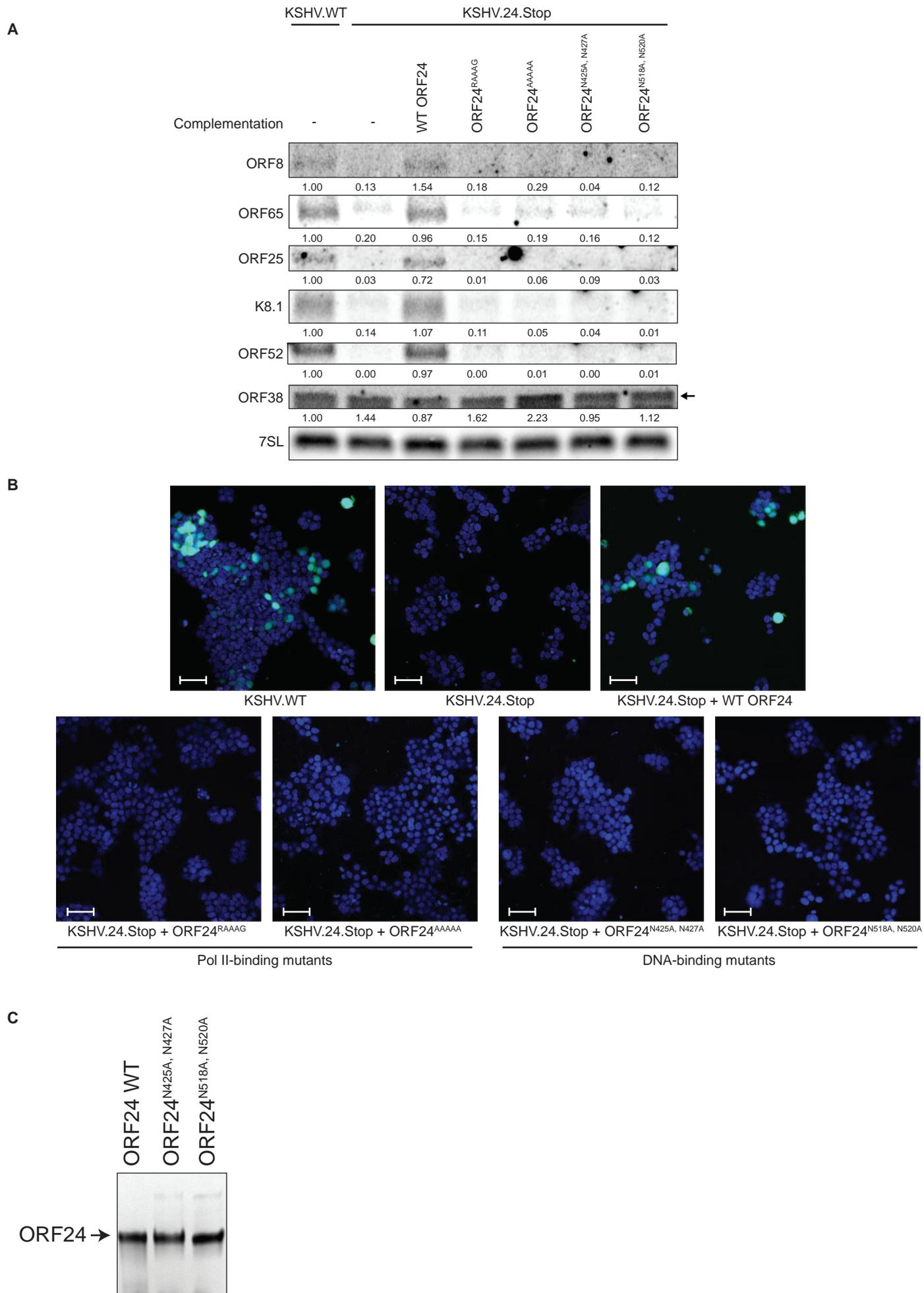


Figure S5

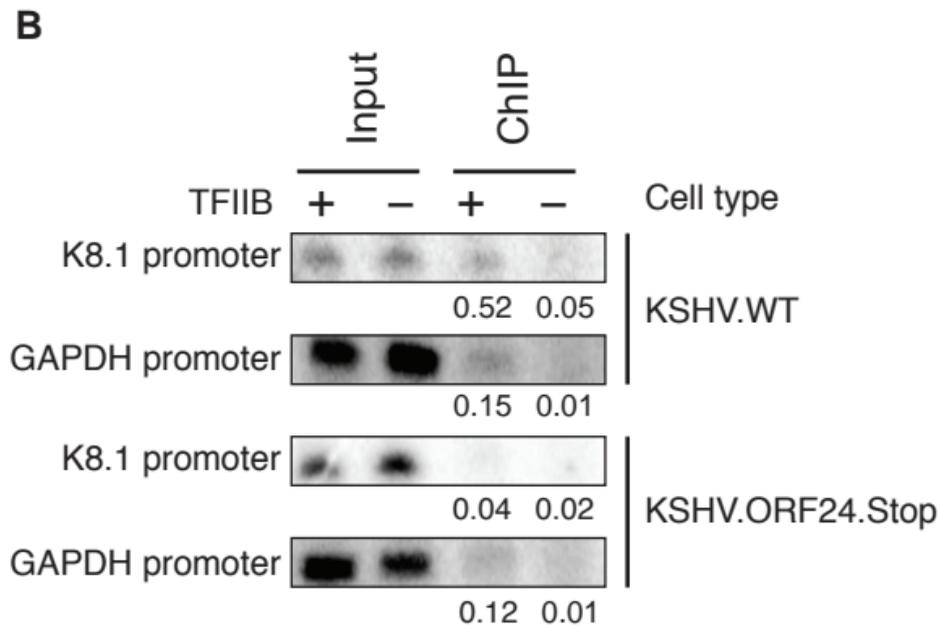
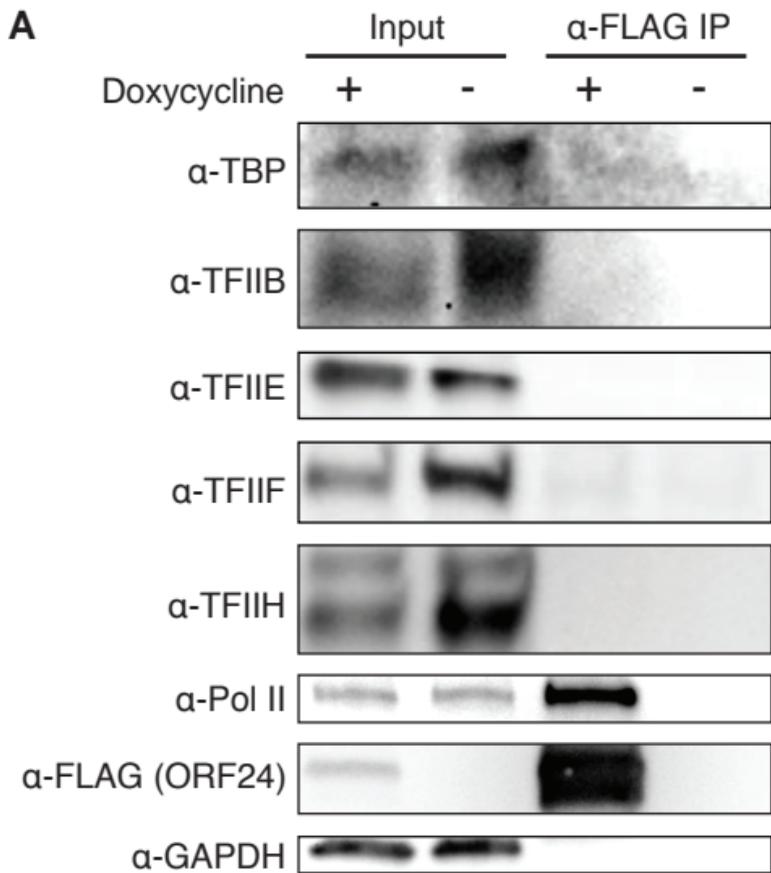


Figure S6

1 **SUPPLEMENTAL FIGURE LEGENDS**

2 **Figure S1 (associated with Figure 1): Protein expression levels and KSHV**
3 **latency protein interactors linked to cancer.**

4 (A) Plasmids expressing strep-tagged KSHV ORFs were transfected into 293T cells
5 and their expression level compared to that in unreactivated (no doxycycline) and
6 reactivated (with doxycycline) KSHV.WT iSLK cells using the indicated antibodies
7 against each viral protein. Viral proteins in 293T cells are slightly larger than in
8 infected cells due to the strep tag.

9 (B) ROC curves comparing the optimal MiST feature weights to CompPASS (Sowa et
10 al., 2009) and SAINT (Choi et al., 2011) scoring algorithms. The high-confidence data
11 set (MiST ≥ 0.7) is indicated with an x.

12 (C) Viral and cellular protein pairs were mapped in three-dimensional space
13 showing the reproducibility, abundance, and specificity features of the MiST score.
14 Interaction pairs with MiST scores ≥ 0.7 are indicated in red.

15 (D) Bar graph showing the percentage of all human proteins, versus the subset that
16 interact with KSHV lytic or latent proteins, that are associated with cancer using the
17 cBioPortal (TCGA) cancer mutation dataset (Cerami et al., 2012). ** Indicates a p-
18 value of < 0.005 as determined by the hyper-geometric test.

19 (E) Network of KSHV latency protein interactions showing enrichment for cellular
20 proteins linked to cancer. The KSHV latency protein-human interactome is shown
21 with viral proteins as gold nodes and associated cellular proteins as red (associated
22 with cancer) or grey (not associated with cancer) nodes. Disease terms from the
23 DisGeNET (Bauer-Mehren et al., 2011) database were manually collapsed to create

24 curated terms for the network. Interactions between cellular proteins present in the
25 interaction network for each individual viral protein are displayed using black
26 edges. Host-host edges were manually curated from CORUM (Ruepp et al., 2010)
27 and the literature.

28

29 **Figure S2 (associated with Figure 2): Full high-confidence KSHV interactome**
30 **network overlaid with all host-host connections and cellular disease-linked**
31 **proteins.**

32 The KSHV-human interactome is shown with viral proteins as gold nodes and
33 associated cellular proteins as pie chart nodes, with the coloring indicating the
34 association of that cellular protein with a particular disease (specified by the colors
35 in the legend). Host proteins without disease association are shown in grey. Disease
36 terms from the DisGeNET (Bauer-Mehren et al., 2011) database were manually
37 collapsed to create curated terms for the network. All known interactions between
38 cellular proteins present in the interactome are displayed using black and grey
39 edges. Host-host edges were manually curated from CORUM (Ruepp et al., 2010)
40 and the literature.

41

42 **Figure S3 (associated with Figure 3): Gene ontology and domain enrichment**
43 **for the KSHV interactome.**

44 (A) Heat map depicting the enrichment of biological processes of cellular proteins
45 interacting with KSHV baits via a GO term analysis. The color scale corresponds to
46 the significance of the bait-term association q-values (p-values adjusted for FDR).

47 (B) Heat map representing the enrichment q-values for the domains found in the
48 interacting proteins of KSHV.

49

50 **Figure S4 (associated with Figure 4): ORF24 interacts with Pol II in a DNA-**
51 **independent manner and the interaction is conserved across ORF24**
52 **orthologs.**

53 (A) Pol II interaction is not a conserved feature of viral transcription factors. Lysates
54 of 293T cells transfected with either empty vector or plasmids expressing the strep-
55 tagged KSHV transcription factors K8 α or ORF50 (RTA) were subjected to α -strep IP
56 and Western blotted with antibodies against strep and Pol II. Input lanes represent
57 5% of the lysate used for IP.

58 (B) The interaction between ORF24 and Pol II is DNA independent. Lysates were
59 prepared as described above, but were treated with DNase prior to α -FLAG IP. Input
60 lanes represent 5% of the lysate used for IP. GAPDH was used as a loading and IP
61 specificity control.

62 (C) Percent identity matrix for ORF24 orthologs.

63 (D-F) ORF24 orthologs from other herpesviruses all interact with Pol II. 293T cells
64 were transfected with plasmids expressing FLAG mORF24 from MHV68 (E), FLAG
65 BcRF1 from EBV (F), or strep UL87 from HCMV (G). The viral proteins were then
66 subjected to IP with the indicated antibody and their interaction with Pol II was
67 monitored by Western blotting with an antibody against RPB1. Input lanes
68 represent 5% of the lysate used for IP. Where indicated, RPS6, GAPDH, and tubulin
69 served as loading and IP specificity controls.

70 (G) Deletion of ORF24 from KSHV prevents expression of late but not early genes.
71 Western blots of lysates from iSLK cells lytically infected with WT KSHV, KSHV
72 bearing a stop codon at the 5' end of ORF24 (KSHV.24.Stop), or a mutant rescue
73 KSHV in which the stop mutation was repaired to WT (KSHV.MR). SOX (ORF37) is a
74 KSHV protein expressed with early kinetics, whereas K8.1 is a KSHV late protein. Pol
75 II serves as a loading control.

76 (H) ORF24 is not required for viral DNA amplification. qRT-PCR measurements of
77 the DNA levels of the KSHV viral genome in iSLK cells containing WT KSHV,
78 KSHV.24.Stop, or KSHV.MR 24 and 48 hr after induction into the lytic cycle.

79 (I) Lysates of the KSHV.24.Stop iSLK cell lines transduced with the indicated WT or
80 mutant ORF24 for the complementation assay were Western blotted with anti-FLAG
81 antibodies to confirm equivalent expression of the ORF24 proteins. Pol II serves as a
82 loading control.

83

84 **Figure S5 (associated with Figure 5): ORF24 mutants unable to bind either**
85 **DNA or Pol II are defective for late gene expression and virion production.**

86 (A) RNA harvested from lytically reactivated KSHV.WT and KSHV.24.Stop iSLK cells
87 complemented with the indicated WT or mutant ORF24 was Northern blotted using
88 strand specific primer probes against the late genes ORF8, ORF65, ORF25, K8.1, and
89 ORF52, the ORF38 early gene, or 7SL (as a loading control).

90 (B) Both the DNA binding and Pol II binding activities of ORF24 are required for
91 production of infectious progeny virions. KSHV.WT and KSHV.24.Stop iSLK cells
92 complemented with WT ORF24, ORF24-Pol II binding mutants, or DNA-binding

93 mutants were lytically reactivated for 48 hr and supernatant from these cells was
94 collected and transferred to recipient 293T cells. After 24 hr, the recipient cells were
95 monitored for GFP expression, as infectious virus constitutively expresses GFP. DNA
96 is stained with DAPI (blue). A minimum of 30 fields were monitored for each
97 experiment, with a single representative field shown. No GFP positive cells were
98 detected in any field of recipient cells containing supernatant from infected cells
99 lacking WT ORF24. Scale bar = 50 μ m.

100 (C) Coomassie stained gel showing the purity of the ORF24 protein used in EMSA
101 assays.

102

103 **Figure S6 (associated with Figure 6): ORF24 interacts selectively with Pol II**
104 **and is needed for late gene PIC assembly.**

105 (A) Lysates of iSLK cells either latently (- doxycycline) or lytically (+ doxycycline)
106 infected with KSHV.FLAG.ORF24 were subjected to α -FLAG IP and Western blotted
107 with antibodies to the indicated general transcription factors or GAPDH as a control.
108 Input lanes represent 5% of the lysate used for IP.

109 (B) Chromatin from iSLK cells lytically infected with KSHV.WT or KSHV.24.Stop was
110 isolated and subjected to ChIP using antibodies against TFIIB. Co-precipitating DNA
111 was detected by PCR using radiolabeled nucleotides and primers specific for the
112 indicated promoter. Experiments were repeated at least three times and a
113 representative image is presented with the ChIPs quantified against input samples.

114

115 **Table S1: MS data for all proteins detected in the KSHV interactome and ROC**
116 **data, and attempted interactome validations, related to Figures 1 and 2.**

117 MiST-abundance, MiST-reproducibility, and MiST-specificity refer to the individual
118 component scores for each feature. The MiST composite score is the weighted
119 combination of the specificity and reproducibility scores. The unique peptide
120 average is the sum of unique prey peptides across all pulled down baits, divided by
121 the number of replicates. The “high confidence interactions” tab contains all
122 interactors with MiST scores ≥ 0.7 while the “All detected interactions” tab contains
123 all collected data regardless of score. In the "ROC data" tab are all of the data points
124 for the ROC curves. RAS refers to the order of the Reproducibility, Abundance, and
125 Specificity weights for each curve. Scores above threshold refers to the number of
126 interactions with MiST scores at or above the listed MiST threshold value. The
127 "validated interactions" tab refers to the attempted validations by co-IP done for
128 high confidence interaction partners identified by the interactome. Positive
129 interactions are indicated by +; the absence of a detected interaction by co-IP is
130 indicated by -. Interaction partners were epitope-tagged expression plasmids
131 unless otherwise indicated.

132

133 **Table S2: Previously described interactions for KSHV viral proteins, related to**
134 **Figure 1.**

135 Prey proteins found in the high (H) confidence data set correspond to a MiST score
136 of ≥ 0.7 . Proteins found below the cutoff are labeled low (L) while proteins absent
137 from the MS, regardless of score are labeled not detected (ND).

138

139 **Table S3: Disease network dictionary, related to Figures 1, S1, and S2.**

140 Disease terms from the DisGeNET database were manually collapsed to create
141 curated terms for the Figures S1 and S2 networks. The first tab (Disease term
142 dictionary) contains the dictionary and the second tab (Disease term summary)
143 defines which disease terms were collapsed together to generate the new term.

144

145 **Table S4: KSHV-HIV-1 high confidence overlapping proteins, related to Figure**
146 **1.**

147 Cellular proteins interacting with at least one HIV-1 and KSHV protein are divided
148 by functional category. The viral proteins interacting with the cellular protein are
149 written after in parentheses with the KSHV protein(s) first in black and the HIV-1
150 protein(s) second in orange.

151

152 **Table S5: Host-host edges in interactome network depictions, related to**
153 **Figures 2 and S2.**

154 Host-host edges depicted in Figure 2 were restricted to host protein pairs that
155 interact with the same KSHV bait (listed in the “Hu-Hu interactions in Fig 2” tab). In
156 Figure S2, all host-host edges, including host protein pairs that interact with
157 different KSHV baits, were shown (listed in the “Hu-Hu interactions in Fig S2” tab).

158

159 **Table S6: Gene ontology term curation, related to Figures 3 and S3.**

160 Gene ontology terms from the GO biological processes database were manually
161 collapsed to create curated terms for the heat map. The first tab (Dictionary for GO
162 terms) contains the dictionary and the second tab (GO terms in dictionary) defines
163 which processes were collapsed together to generate the term.

164

165 **Table S7: Domain and GO term summary, related to Figures 3 and S3.**

166 Cellular location refers to the location on the Figure 3B cell diagram. The ORFs to
167 annotate in the various locations were determined by significant enrichment in the
168 listed GO terms or domains.

Table S2: Previously described interactions for KSHV viral proteins, related to Figure 1.

ORF	Interaction Partner	Source	Not Detected/High/Low
K2	IL6ST	(Li et al., 2001)	H
K2	VKORC1	(Chen et al., 2012)	ND
K9	EP300	(Burysek et al., 1999)	H
K9	CREBBP	(Lin et al., 2001)	H
K9	NDUFA13	(Seo et al., 2002)	ND
K9	IRF3	(Lin et al., 2001)	ND
K9	IRF7	(Lin et al., 2001)	ND
K10	RBPJ	(Heinzelmann et al., 2010)	ND
K10	PABPC	(Kanno et al., 2006)	ND
K12A	CYTH1	(Kliche et al., 2001)	ND
K12B	MAPKAPK2	(McCormick and Ganem, 2005)	ND
ORF45	SIAH1	(Abada et al., 2008)	H
ORF45	SIAH2	(Abada et al., 2008)	H
ORF45	RSK1	(Kuang et al., 2008)	H
ORF45	RSK2	(Kuang et al., 2008)	H
ORF45	MAPK1	(Kuang et al., 2009)	H
ORF45	KIF3A	(Sathish et al., 2009)	ND
ORF45	IRF7	(Zhu et al., 2002)	ND
ORF50	PARP1	(Gwack et al., 2003)	L
ORF50	POU2F1	(Carroll et al., 2007)	ND
ORF50	RBPJ	(Liang et al., 2002)	ND
ORF57	ALYREF	(Malik et al., 2004)	L
ORF57	WIBG	(Boyne et al., 2010)	L
ORF57	NUP62	(Malik et al., 2012)	ND
ORF57	FYTTD1	(Jackson et al., 2011)	ND
ORF71	IKBKG	(Liu et al., 2002)	H
ORF71	IKBKB	(Liu et al., 2002)	H
ORF71	CHUK	(Liu et al., 2002)	H
ORF71	TRAF2	(Guasparri et al., 2006)	ND
ORF72	CDK2	(Platt et al., 2000)	H
ORF72	CDK5	(Platt et al., 2000)	H
ORF72	CDK6	(Platt et al., 2000)	H
ORF72	CDK4	(Platt et al., 2000)	ND
ORF73	BRD2	(Platt et al., 1999)	L
ORF73	KDM3A	(Kim et al., 2013)	H
ORF73	H2A	(Barbera et al., 2006)	H

ORF73	H2B	(Barbera et al., 2006)	L
ORF73	RPA1	(Shamay et al., 2012)	H
ORF73	RPA2	(Shamay et al., 2012)	H
ORF73	DAXX	(Murakami et al., 2006)	H
ORF73	MECP2	(Krithivas et al., 2002)	H
ORF73	TP53	(Friborg et al., 1999)	L
ORF73	TRF1	(Shamay et al., 2012)	L
ORF73	XPC	(Shamay et al., 2012)	H
ORF73	ANG	(Paudel et al., 2012)	ND
ORF73	ANXA2	(Paudel et al., 2012)	ND
ORF73	BRD4	(Ottinger et al., 2006)	ND
ORF73	DEK	(Krithivas et al., 2002)	ND
ORF73	CBX5	(Lim et al., 2003)	ND
ORF73	VHL	(Cai et al., 2006)	ND
ORF73	RB1	(Radkov et al., 2000)	ND
ORF73	CENPF	(Shamay et al., 2012)	ND
ORF73	YIPF3	(Shamay et al., 2012)	ND
ORF73	HMGA1	(Shamay et al., 2012)	ND
ORF73	HMGA2	(Shamay et al., 2012)	ND
ORF73	PYGO2	(Shamay et al., 2012)	ND
ORF73	PPP2R2A	(Shamay et al., 2012)	ND
ORF73	TIP60	(Shamay et al., 2012)	ND
ORF73	CUL5	(Cai et al., 2006)	ND
ORF73	RBX1	(Cai et al., 2006)	ND
ORF73	TCEB1	(Cai et al., 2006)	ND
ORF73	CBP	(Lim et al., 2001)	ND

Table S4: KSHV-HIV-1 high confidence overlapping proteins, related to Figure 1.

Term	Cellular protein (KSHV protein, HIV protein)
Endoplasmic reticulum (13)	GANAB (K2, Gp120 and Gp160); ERLEC1 (K8.1, Gp41 and Gp160); RCN1 (K1, Pol); DNAJC7 (K12C, Vif); CALR (K1, Gp160); CANX (K2, Gp160); PDIA4 (ORF47, IN and Pol); PRKCSH (K2, Gp120 and Gp160); PLOD1 (ORF21, IN); PDIA3 (ORF47, Gp41); DNAJB11 (K1, Gp120 and Gp160); P4HB (ORF47, Gp160); SDF2L1 (K1, Gp120 and Gp160)
Mitochondria (9)	ATAD3A (K1, Vpu); TOMM40 (ORF56, Gp41); TMEM55B (K8.1, Vpu); TMEM11 (K8.1, Vpr); MTCH2 (K8.1, Vpr); MRS2 (ORF11, Rev); COX4IL (K8.1, Gp41 and Gp160); HADHB (K2 and K8.1, Vpu); NDUFB10 (K8.1, Gp41)
DNA replication (4)	POLE (K8.1, Pr); RECQL (K10, Pol); PRMT1 (K8.1, Gag and NC); MSH2 (ORF62, Pr)
Signaling (4)	FBN2 (K10.6, Tat); GRB2 (ORF39, RT); ATXN10 (ORF4, Vpu); PRDX4 (ORF2, Gp41)
Cell cycle (3)	CDKN2A (ORF72, Nef); APP (ORF47, Gp160); TBRG4 (ORF4, Vpu)
Cellular organization (3)	DSC3 (K14, Gp160); EPPK1 (K10, Pr); SDCCAG8 (ORF21, MA)
Golgi apparatus (3)	FUT8 (K8.1, Gp160); GOLPH3 (ORF16, Vpr); LMAN1 (ORF27, Gp41 and Gp160)
Lipid transport (3)	OSBPL6 (ORF46, Pr); OSBPL3 (ORF46, Pol); SGPL1 (K8.1, Vpu)
Protein modification (3)	DPY19L3 (K8.1, Vpr); ITM2B (K14, Gp160); SDF2 (K1, Gp120 and Gp160)
E3 (2)	HERC2 (ORF73, Pr); UBR2 (K8.1, Vif)
RNA processing (2)	YTHDF3 (ORF10, NC); THRAP3 (ORF21, Gag)
E2 (1)	UBE2O (ORF57, Tat)
Import/export (1)	XPO4 (ORF4, Vpu)
Transcription (1)	PHB2 (K1, Gp41)

EXTENDED EXPERIMENTAL PROCEDURES

Construct generation

Plasmids expressing ORF63 (Gregory et al., 2011) and ORF64 (Gonzalez et al., 2009) were kindly provided by Blossom Damania and were used for subcloning. Cloning of MHV68 ORF24 and EBV BcRF1 was carried out in the same manner described for KSHV genes using RNA from MHV68 infected 3T3 cells and EBV infected Akata cells reactivated with anti-human IgG (Takada and Ono, 1989). CMV UL87 was cloned using Towne BAC DNA as a template. ORF24 DNA binding and Pol II binding mutants were made by site-directed mutagenesis. ORF24 for expression in BJAB and iSLK.219 cells was PCR amplified and subcloned into the pLPCX retroviral vector (Clontech) using 5' BglII and 3' Hind III restriction sites. WT and mutant versions of ORF24 were cloned into the pQCXIN retroviral vector (Clontech) using 5' AgeI and 3' BamHI restriction sites.

Transductions

Briefly, pLPCX or pQCXIN retroviral plasmids were co-transfected with VSV G into low passage Phoenix 293 cells. Supernatant containing viral particles was collected 48 hr later, filtered, complemented with 8 µg/mL polybrene and centrifuged onto target cells. Transfected BJABs transduced with pLPCX constructs were selected for two weeks with 1 µg/mL puromycin (Millipore).

Affinity purification and Western blotting

Cells were incubated in lysis buffer [0.5% NP-40, 150 mM NaCl, 50 mM Tris pH 7.4, 1 mM EDTA and protease inhibitors (Roche)]. Equivalent amounts of clarified lysate were added to 30 mL pre-washed Strep-Tactin beads (IBA) or FLAG beads (Sigma) and allowed to bind for 1-3 hr, washed 3 times with lysis buffer lacking protease inhibitors and containing 0.05% NP-40, and washed 3 additional times in lysis buffer lacking NP-40. Complexes were eluted using desthiobiotin (IBA) or 3X FLAG peptide (Elim BioPharm) for strep or FLAG tags, respectively. The elutions were divided into thirds and analyzed by Western blotting to confirm protein expression with α -FLAG (ORF24 in iSLK.219 cells) or α -strep (all MS in 293T cells) antibodies, silver stain, and mass spectrometry.

Co-immunoprecipitations for experiments other than MS were performed similarly, except with the omission of the final washes in buffer lacking NP-40. Western blots were performed using the following antibodies: rabbit α -FLAG (1:5000; Sigma), mouse α -FLAG (clone M2; 1:5000; Sigma), mouse α - β -tubulin (clone TUB 2.1; 1:3000; Sigma), mouse α -strep (1:3000; Qiagen), strep direct HRP conjugate antibody (1:10,000; Novagen), rabbit α -RNA Pol II (N20; 1:5000; Santa Cruz Biotechnologies), rabbit α -V5 (1:3000; Invitrogen), rabbit α -TFIIB (clone SI-1; 1:1,000; Santa Cruz Biotechnologies), mouse α -TBP (clone 1TBP18; 1:1000; Abcam), rabbit α -TFIIE (clone C-21; 1:1,000; Santa Cruz Biotechnologies), rabbit α -TFIIF RAP74 (clone N-16; 1:1,000; Santa Cruz Biotechnologies), rabbit α -TFIIH p89 (clone S-19; 1:1,000; Santa Cruz Biotechnologies), rabbit α -GAPDH (1:1,000; Abcam), mouse α -S6 ribosomal protein (clone 54D2; 1:1000; Cell Signaling), rat α -Pol II serine 5 (clone 3E8; 1:10,000; Millipore), rat α -Pol II serine 7 (clone 4E12; 1:10,000;

Millipore), goat α -rat HRP secondary antibody (1:5000; Cell Signaling), and goat α -mouse and goat α -rabbit HRP secondary antibodies (1:5000; Southern Biotech).

For nuclease treatment of whole cell lysate, lysis buffer without EDTA was used [0.5% NP-40, 150 mM NaCl, 50 mM Tris pH 7.4, and protease inhibitors (Roche)]. To 500 μ g of cell lysate, 2 μ L of DNase I (NEB), 2 μ L of micrococcal nuclease, and micrococcal nuclease buffer (NEB) were added and allowed to incubate for 1 hr at 4°C. Following incubation, 10 mM EDTA was added to inactivate the nucleases. Immunoprecipitation was then carried out as described.

Mass spectrometry

Affinity purified protein eluates were first denatured and reduced in 2 M urea, 10 mM NH_4HCO_3 , and 2 mM DTT for 30 min at 60°C, then alkylated with 2 mM iodoacetamide for 45 min at room temperature. Trypsin (Promega) was added at a 1:100 enzyme: substrate ratio and digested overnight at 37°C. Following digestion, samples were concentrated using C18 ZipTips (Millipore) according to the manufacturer's specifications.

Samples were injected onto a pre-column (2 cm x 100 μ m I.D. packed with ReproSil Pur C18 AQ 5 μ m particles) in 0.1% formic acid and then separated with a 1 hr gradient from 5% to 30% acetonitrile in 0.1% formic acid on an analytical column (10 cm x 75 μ m I.D. packed with ReproSil Pur C18 AQ 3 μ m particles). The mass spectrometer collected data in a data-dependent fashion, collecting one full scan followed by 20 collision-induced dissociation MS/MS scans of the 20 most intense peaks from the full scan. Dynamic exclusion was enabled for 30 seconds

with a repeat count of 1. The resulting raw data was matched to protein sequences by the Protein Prospector algorithm (Clauser et al., 1999). Data were searched against a database containing SwissProt Human protein sequences (downloaded April 13, 2012) and viral sequences, concatenated to a decoy database where each sequence was randomized in order to estimate the false positive rate. The searches considered a precursor mass tolerance of 1 Da and fragment ion tolerances of 0.8 Da, and considered variable modifications for protein N-terminal acetylation, protein N-terminal acetylation and oxidation, glutamine to pyroglutamate conversion for peptide N-terminal glutamine residues, protein N-terminal methionine loss, protein N-terminal acetylation and methionine loss, and methionine oxidation, and constant modification for carbamidomethyl cysteine. Prospector data was filtered using a maximum protein expectation value of 0.01 and a maximum peptide expectation value of 0.05.

Disease association analysis

First, all 20,264 proteins in the human reference proteome were mapped to gene-disease associations in DisGeNET (v.1) using Uniprot accession codes. All proteins in the reference proteome were labeled as 'cancer-associated', 'other disease associated' or 'none' based on their DisGeNET disease annotations. Cancer association was called using a regular expression search with tumor and cancer-related keywords. Every protein with at least one cancer-associated term was labeled as a cancer-associated protein. Second, the reference proteome was divided in three sets: all proteins, latent KSHV ORF (K1, K2, K12A, K12B, K12C, ORF71,

ORF72 and ORF73) interacting proteins or lytic KSHV ORF interacting proteins (remaining). Using this partitioning, relative fractions of proteins with cancer-associations or other disease associations were computed and the significance of the observed counts was computed with the hyper-geometric test. The nodeCharts plugin for Cytoscape was used to create disease association pie charts (Figure S2).

Comparison to HIV-1 AP-MS data set

The significance of observing an overlap of 52 proteins between the previously published data set of 292 unique HIV host factors characterized by AP-MS in HEK-293T cells and the 556 unique KSHV factors described here given the human reference proteome (n=20,264) was computed by the hyper-geometric test. The 52 overlapping proteins were annotated with GO annotations.

KSHV Mutagenesis

Electrocompetent GS1783 *E. coli* cells containing WT KSHV BAC16 and transiently expressing *gam*, *bet* and *exo* were electroporated with a kanamycin resistance cassette containing an I-SceI restriction site and flanked by homologous arms to the region to be mutated. After sequence verifying the insertion of the resistance gene and the mutation, the second step of recombination was performed to remove the cassette, leaving kanamycin sensitive clones that were screened by replica plating. Pulsed-field gel electrophoresis (PFGE) of NheI digested BAC DNA was used to verify BAC integrity. Purified BAC16 DNA was transfected into low passage iSLK cells using PolyJet (SignaGen) and BAC16-containing cells were selected with 400

$\mu\text{g}/\text{mL}$ of hygromycin. Individual clones were amplified and tested for their ability to reactivate by Western blotting and RT-qPCR for early viral gene products (data not shown).

Northern blotting

RNA was collected and purified from lytically reactivated BAC16-containing iSLK cells using Trizol (Invitrogen). Blots were hybridized with oligonucleotide probes end-labeled with $\gamma\text{-}^{32}\text{P}$ ATP using T4 PNK (NEB), then scanned using a PharosFX Imager System (BioRad).

Infectious virion production assay

BAC16-containing iSLK cells were reactivated for three days with sodium butyrate and doxycycline. Supernatant was collected and passed through a $0.45\ \mu\text{m}$ filter. Filtered supernatant containing $8\ \mu\text{g}/\text{mL}$ polybrene was added to 293T cells plated on poly-L-lysine-treated glass coverslips. Twenty-four hr later, slides were washed with PBS, fixed in 4% formaldehyde, washed again with PBS, and mounted on slides with DAPI (Vector Labs). Slides were imaged with an LSM 710 laser scanning confocal microscope (Zeiss). BAC16 constitutively expresses GFP, thus, GFP positive cells indicated the production and transfer of infectious virions.

Chromatin immunoprecipitation

ChIPs were performed with $10\ \mu\text{g}$ of chromatin and $2.5\ \mu\text{g}$ of the following antibodies: rabbit anti-RNA Pol II (N20; Santa Cruz Biotechnologies), rabbit anti-

TFIIH p89 (clone S-19; Santa Cruz Biotechnologies), rabbit anti-TFIIB (clone SI-1; Santa Cruz Biotechnologies), mouse anti-TBP (clone 1TBP18; Abcam) and mouse anti-FLAG (clone M2; Sigma).

Primer	Sequence (5'-3')	Orientation F: Forward R: Reverse	Use
K2 (vIL-6) (BamHI)	GCTCGGATCCATGTGCTGGTTCAAGTTGTGGTC	F	Cloning
K2 (vIL-6) (NotI)	TCGAGCGGCCGCCCTGACTTATCGTGGACGTCAGGAG	R	Cloning
K3 (EcoRI)	GGTGGAATTCATGGAAGATGAGGATGTTCTGTCTG	F	Cloning
K3 (NotI)	TCGAGCGGCCGCCCTGAATGAAACATAAGGGCAGACGAAAC	R	Cloning
K4 (EcoRI)	GGTGGAATTCATGGACACCAAGGGCATCCT	F	Cloning
K4 (NotI)	TCGAGCGGCCGCCCTCCGCGAGCAGTGACTGG	R	Cloning
K4.1 (KpnI)	GCTTGGTACCATGTGGAGCATGTGCTGGG	F	Cloning
K4.1 (NotI)	TCGAGCGGCCGCCCGAGGGGCATAACCCTTTACC	R	Cloning
K4.2 (EcoRI)	GGTGGAATTCATGCAAATTAGCTTTGCCGAAGTTCT	F	Cloning
K4.2 (NotI)	TCGAGCGGCCGCCCTGATTGAAGCCCAGGCGAC	R	Cloning
K5 (EcoRI)	GGTGGAATTCATGGCGTCTAAGGACGTAGAAGAG	F	Cloning
K5 (NotI)	TCGAGCGGCCGCCCTCCACCGTTGTTTTTGGATGATTTTTTC	R	Cloning
K6 (EcoRI)	GGTGGAATTCATGGCCCCGTCCACG	F	Cloning
K6 (NotI)	TCGAGCGGCCGCCCGAAGCTATGGCAGGCAGC	R	Cloning
K7 (EcoRI)	GGTGGAATTCATGGGAACACTGGAGATAAAAAGGGG	F	Cloning
K7 (NotI)	TCGAGCGGCCGCCCGACAACCTGGCCTGGAGATTG	R	Cloning
K8 α (kBZIP) (EcoRI)	AGTGTGGTGGAAATTCATGCCAGAATGAAGGACATACCT	F	Cloning
K8 α (kBZIP) (NotI)	CCCTCGAGCGGCCGCCCTGAACATGGTGGGAGTGGC	R	Cloning
K8 β (EcoRI)	GGTGGAATTCATGCCAGAATGAAGGACATACCT	F	Cloning
K8 β (NotI)	TCGAGCGGCCGCCCGATACCTGCTGCAGCTGTCT	R	Cloning
K8.1 (BamHI)	GCTCGGATCCATGAGTTCCACACAGATTCGCAC	F	Cloning
K8.1 (NotI)	TCGAGCGGCCGCCCTGACACTATGTAGGGTTTCTTACGCC	R	Cloning
K9 (EcoRI)	GGTGGAATTCATGGACCCAGGCCAAAGACC	F	Cloning
K9 (NotI)	TCGAGCGGCCGCCCTGATTGCATGGCATCCCATAACG	R	Cloning
K10 (EcoRI)	GGTGGAATTCATGGGGTCTCTGGGACG	F	Cloning
K10 (NotI)	TCGAGCGGCCGCCCTCCTGTAGACTATCCCAAATGGAGC	R	Cloning
K10.5	GGTGGAATTCATGTACCACGTGGGACAGGAG	F	Cloning

(EcoRI)			
K10.5 (NotI)	TCGAGCGGCCGCCCTGAGTCATCACATGTAACGAAACGCA	R	Cloning
K10.6 (EcoRI)	GGTGAATTCATGGCGGGACGCAGG	F	Cloning
K10.6 (NotI)	TCGAGCGGCCGCCCTGACCTTGGTCTTCTCCGATGC	R	Cloning
K11 (EcoRI)	GGTGAATTCATGCACAGTTTGTGTTTTTTGAAGAGCC	F	Cloning
K11 (NotI)	TCGAGCGGCCGCCCTGAGTCTCTGTGGTAAAATGGGGC	R	Cloning
K11.1 (EcoRI)	GGTGAATTCATGCCTCGCTACACGGAGTC	F	Cloning
K11.1 (NotI)	TCGAGCGGCCGCCCTGAGTCTCTGTGGTAAAATGGGGC	R	Cloning
K13 (vFLIP) (EcoRI)	GGTGAATTCATGGCCACTTACGAGGTTCTCTG	F	Cloning
K13 (vFLIP) (NotI)	TCGAGCGGCCGCCCGATGGTGTATGGCGATAGTGTTG	R	Cloning
K14 (vOX2) (EcoRI)	GGTGAATTCATGATACACACATTTTTTGATTGTCCCGG	F	Cloning
K14 (vOX2) (NotI)	TCGAGCGGCCGCCCTCCCTGGGTGGATAGGGG	R	Cloning
ORF2 (EcoRI)	GGTGAATTCATGGATCCTACACTTTACTGTGTAGTTGC	F	Cloning
ORF2 (NotI)	TCGAGCGGCCGCCCTGACGAAGTCTCACTGAAGGGC	R	Cloning
ORF4 (EcoRI)	GGTGAATTCATGGCCTTTTTAAGACAAACACTGTGG	F	Cloning
ORF4 (NotI)	TCGAGCGGCCGCCCGAACGAAAGAACAGATAGTGAAATAAG GTAATCA	R	Cloning
ORF6 (SSB) (BamHI)	GCTCGGATCCATGGCGCTAAAGGGACCACA	F	Cloning
ORF6 (SSB) (NotI)	TCGAGCGGCCGCCCGACAAATCCAGGTCAGAGAGCA	R	Cloning
ORF7 (EcoRI)	GGTGAATTCATGGCAAAGGAACTGGCGG	F	Cloning
ORF7 (NotI)	TCGAGCGGCCGCCCGAGACCTGGGAGTCATTGTGG	R	Cloning
ORF8 (gB) (EcoRI)	AAGAATTCATGACTCCAGGTCTAGATTGGC	F	Cloning
ORF8 (gB) (NotI)	TCGAGCGGCCCGCTCCCTCCCCGTTTCCG	R	Cloning
ORF9 (Pol) (EcoRI)	GGTGAATTCATGGATTTTTTCAATCCATTTATCGACCCAAC	F	Cloning
ORF9 (Pol) (NotI)	TCGAGCGGCCGCCCGAGGGCGTGGGAAAAGTC	R	Cloning
ORF10 (RIF)	GGTGAATTCATGCAGACAGAGGCAACGTTC	F	Cloning

(EcoRI)			
ORF10 (RIF) (NotI)	TCGAGCGGCCGCCCTCCCGATTGCATGGGTTTCCT	R	Cloning
ORF11 (AflII)	TAAACTTAAGATGGCGCAGGAGTCAGAGC	F	Cloning
ORF11 (NotI)	TGCAGAATTCGGAAGTCCGTCGGTGG	R	Cloning
ORF16 (EcoRI)	GGTGAATTCATGGACGAGGACGTTTTGCCT	F	Cloning
ORF16 (NotI)	TCGAGCGGCCGCCCTGATCTCCTGCTCATCGCGAC	R	Cloning
ORF17 (Protease) (EcoRI)	GGTGAATTCATGAGCCTCCTAAGCCCCG	F	Cloning
ORF17 (Protease) (NotI)	TCGAGCGGCCGCCCGACTGCTTGTTCAGGAGCTC	R	Cloning
ORF17.5 (Scaffold) (EcoRI)	GGTGAATTCATGAACAGCTCTGGTCAAGAGGAT	F	Cloning
ORF17.5 (Scaffold) (NotI)	TCGAGCGGCCGCCCGACTGCTTGTTCAGGAGCTC	R	Cloning
ORF18 (EcoRI)	GGTGAATTCATGCTCGGAAAATACGTGTGTGAGA	F	Cloning
ORF18 (NotI)	TCGAGCGGCCGCCCTGAAACCGGTTGTTGTTAAACG	R	Cloning
ORF20 (EcoRI)	GGTGAATTCATGTACGAGGTTTTTACAGACTTTCCC	F	Cloning
ORF20 (NotI)	TCGAGCGGCCGCCCTCCTGGACCTGAACAAGCCG	R	Cloning
ORF21 (TK) (EcoRI)	GGTGAATTCATGGCAGAAGGCGTTTTGG	F	Cloning
ORF21 (TK) (NotI)	TCGAGCGGCCGCCCGAGACCCTGCATGTCTCCT	R	Cloning
ORF22 (gH) (BamHI)	GCTCGGATCCATGCAGGGTCTAGCCTTCTTGG	F	Cloning
ORF22 (gH) (NotI)	TCGAGCGGCCGCCCGAATAAAGGATGGAAAACAGTCTGTAA AGAAA	R	Cloning
ORF23 (BamHI)	GCTCGGATCCATGTTACGAGTTCCGGACGTGA	F	Cloning
ORF23 (NotI)	TCGAGCGGCCGCCCTGAGACGGTCAATAAAGCGTAGATTTTT AAAAG	R	Cloning
ORF24 (BamHI)	GCTCGGATCCATGGCAGCGCTCGAGGG	F	Cloning
ORF24 (NotI)	TCGAGCGGCCGCCCTGAGACCAGCGGACGGAC	R	Cloning
ORF25 (MCP)	AGTGTGGTGAATTCATGGAGGCGACCTTGGAGC	F	Cloning

(EcoRI)			
ORF25 (MCP) (NotI)	CTCCCTCGAGCGGCCGCGGCGAATACACCACCTTGTTC	R	Cloning
ORF26 (EcoRI)	GGTGAATTCATGGCACTCGACAAGAGTATAGTGG	F	Cloning
ORF26 (NotI)	TCGAGCGGCCGCCCTGAGCGTGGGGAATACCAACA	R	Cloning
ORF27 (EcoRI)	GGTGAATTCATGGCGTCATCTGATATTCTGTCCG	F	Cloning
ORF27 (NotI)	TCGAGCGGCCGCCCTGATTTAAAATTTAGAATCAAGGGAGGG GTG	R	Cloning
ORF28 (EcoRI)	GGTGAATTCATGAGCATGACTTCCCCGTCT	F	Cloning
ORF28 (NotI)	TCGAGCGGCCGCCCGAATCTGGCATGTATATTGTACGGTAG G	R	Cloning
ORF29a (EcoRI)	GGTGAATTCATGCTGCTCAGCCGTAC	F	Cloning
ORF29a (NotI)	TCGAGCGGCCGCCCTGAAGGCCCTGGGCTTACG	R	Cloning
ORF29b (EcoRI)	GGTGAATTCATGCTTCAGAAAGACGCCAAGC	F	Cloning
ORF29b (NotI)	TCGAGCGGCCGCCCTGATTGTGGGGATATGGGCTTGT	R	Cloning
ORF30 (EcoRI)	GGTGAATTCATGGGTGAGCCAGTGGATCC	F	Cloning
ORF30 (NotI)	TCGAGCGGCCGCCCTCCTTTCGCACCGGTGTCTAG	R	Cloning
ORF31 (EcoRI)	GGTGAATTCATGTCACAAAACAGAAAGACTCTGCC	F	Cloning
ORF31 (NotI)	TCGAGCGGCCGCCCGACGTATCTTTCGTTGATAGCATGC	R	Cloning
ORF32 (EcoRI)	GGTGAATTCATGGATGCGCATGCTATCAACG	F	Cloning
ORF32 (NotI)	TCGAGCGGCCGCCCGAGCCATAGCGGCCTCG	R	Cloning
ORF33 (BamHI)	GCTCGGATCCATGGCTAGCCGAGGCG	F	Cloning
ORF33 (NotI)	TCGAGCGGCCGCCCTCCATAAGAACGTAAGCCCAGGG	R	Cloning
ORF34 (EcoRI)	GGTGAATTCATGTTTGCTTTGAGCTCGCTCG	F	Cloning
ORF34 (NotI)	TCGAGCGGCCGCCCTGAGAGTTGGTTGAGTCCATTCTCC	R	Cloning
ORF35 (BamHI)	GCTCGGATCCATGGACTCAACCAACTCTAAAAGAGAGTT	F	Cloning
ORF35 (XhoI)	CTCCCTCGAGTGAGGGAGTTTCAGGGCACA	R	Cloning
ORF36 (EcoRI)	GGTGAATTCATGCGCTGGAAGAGAATGGAGAG	F	Cloning
ORF36 (NotI)	TCGAGCGGCCGCCCTGAGAAAACAAGTCCGCGGG	R	Cloning
ORF37 (SOX)	GGTGAATTCATGGAGGCCACCCCCAC	F	Cloning

(EcoRI)			
ORF37 (SOX) (NotI)	TCGAGCGGCCGCCCGACGGGCTGTGAGGGA	R	Cloning
ORF38 (EcoRI)	GGTGGAATTCATGGGATTTCTCCTATCTATCTGCAAACG	F	Cloning
ORF38 (NotI)	CTCGAGCGGCCGCCCTGAATAAATTGCTTCTTTATTTTTTTT CTTCTTTTTTAATGCG	R	Cloning
ORF39 (gM) (EcoRI)	GGTGGAATTCATGCGCGCTTCAAAGAGCG	F	Cloning
ORF39 (gM) (NotI)	TCGAGCGGCCGCCCGAAATGAATATCATTGCGTTTCGTCCG AT	R	Cloning
ORF40 (PAF) (EcoRI)	GGTGGAATTCATGGCAACGAGCGAAGAAACG	F	Cloning
ORF40 (PAF) (NotI)	TCGAGCGGCCGCCCTCCAGCAGGGACAGTAGGTC	R	Cloning
ORF41 (PAF) (EcoRI)	GGTGGAATTCATGGCCGGGTTTACTCTGAAGG	F	Cloning
ORF41 (PAF) (NotI)	TCGAGCGGCCGCCCTCCAAATAAAGATAAAAAGCCTGGTCCA	R	Cloning
ORF42 (EcoRI)	GGTGGAATTCATGTCCCTGGAAAGGGCCC	F	Cloning
ORF42 (NotI)	TCGAGCGGCCGCCCTGATTTTGAAAAAGGGAAACAATGGGG	R	Cloning
ORF43 (portal) (EcoRI)	GGTGGAATTCATGTTGAGGATGAACCCGGGG	F	Cloning
ORF43 (portal) (NotI)	TCGAGCGGCCGCCCGATGCACTTCCAGGACAAGG	R	Cloning
ORF44 (HEL) (KpnI)	GCTTGGTACCATGGACAGCTCGGAAGGGTG	F	Cloning
ORF44 (HEL) (NotI)	TCGAGCGGCCGCCCTCCGTAGATCAGAGTAGTCTTGGGG	R	Cloning
ORF45 (EcoRI)	GGTGGAATTCATGGCGATGTTTGTGAGGACCT	F	Cloning
ORF45 (NotI)	TCGAGCGGCCGCCCTCCGTCCAGCCACGGC	R	Cloning
ORF46 (EcoRI)	GGTGGAATTCATGGACGCATGGTTGCAACAG	F	Cloning
ORF46 (NotI)	TCGAGCGGCCGCCCTGACTGCTCCAACAGGCC	R	Cloning
ORF47 (gL) (EcoRI)	GGTGGAATTCATGGGGATCTTTGCGCTATTTGC	F	Cloning
ORF47	TCGAGCGGCCGCCCTGATTTTCCCTTTTGACCTGCGTG	R	Cloning

(gL) (NotI)			
ORF48 (EcoRI)	GGTGGAATTCATGGAGGTGTGTATCCAATTCCG	F	Cloning
ORF48 (NotI)	TCGAGCGGCCGCCCTCCATCATACTCATCGTCGGAGC	R	Cloning
ORF49 (EcoRI)	GGTGGAATTCATGACATCGAGAAGGCCCTTAAAG	F	Cloning
ORF49 (NotI)	TCGAGCGGCCGCCCTGATTGTATACTGAACAATGCGTGTTTA CAAT	R	Cloning
ORF50 (RTA) (EcoRI)	AGTGTGGTGAATTCATGAAAGAATGTCCAAGCTTGGTGC	F	Cloning
ORF50 (RTA) (NotI)	CCCTCGAGCGGCCGCCCTCCGTCTCGGAAGTAATTACGCC	R	Cloning
ORF52 (EcoRI)	GGTGGAATTCATGGCCGCGCCAGG	F	Cloning
ORF52 (XhoI)	CTCCCTCGAGTCCGTCATCAACCCCGC	R	Cloning
ORF53 (gN) (EcoRI)	GGTGGAATTCATGACAGCGTCCACGGTGG	F	Cloning
ORF53 (gN) (NotI)	TCGAGCGGCCGCCCGATGCATGGACCACCTCG	R	Cloning
ORF54 (dUTPase) (EcoRI)	GGTGGAATTCATGAACAACCGCCGAGGC	F	Cloning
ORF54 (dUTPase) (NotI)	TCGAGCGGCCGCCCGAAAACCCAGACGACCC	R	Cloning
ORF55 (EcoRI)	GGTGGAATTCATGTGCTCTCCATGGTACACCTG	F	Cloning
ORF55 (NotI)	TCGAGCGGCCGCCCGATGTGGAACCTATCGCGC	R	Cloning
ORF56 (PRI) (EcoRI)	GGTGGAATTCATGGAGACGACATACCGCCG	F	Cloning
ORF56 (PRI) (NotI)	TCGAGCGGCCGCCCTGAACTGGCCAGTCCCACT	R	Cloning
ORF57 (MTA) (EcoRI)	AGTTGAATTCATGGTACAAGCAATGATAGACATGGACA	F	Cloning
ORF57 (MTA) (NotI)	ATTGCGGCCGCTGAAGAAAGTGGATAAAAGAATAAACCTTG TTAAATTTGG	R	Cloning
ORF58 (EcoRI)	GGTGGAATTCATGTGCCGCTGGACAGT	F	Cloning
ORF58 (NotI)	TCGAGCGGCCGCCCTGAGCCAACAACCTTTATTTATTACCGAC AG	R	Cloning
ORF59 (PPF) (EcoRI)	GGTGGAATTCATGCCTGTGGATTTTCACTATGGG	F	Cloning

ORF59 (PPF) (NotI)	TCGAGCGGCCGCCCTCCAATCAGGGGGTTAAATGTGGT	R	Cloning
ORF60 (EcoRI)	GGTGGAATTCATGGATTGAGTTGATCGATTTCTGTATACAAG	F	Cloning
ORF60 (NotI)	TCGAGCGGCCGCCCTCCCAAATCGTCAGTCACACAC	R	Cloning
ORF61 (EcoRI)	GGTGGAATTCATGTCTGTCCGGACATTTTGTGTCAG	F	Cloning
ORF61 (NotI)	TCGAGCGGCCGCCCGACTGACAGACCAGGCACT	R	Cloning
ORF62 (EcoRI)	GGTGGAATTCATGAAGGTGCAGGCTGAAAATGC	F	Cloning
ORF62 (NotI)	TCGAGCGGCCGCCCTGACAGAAACACAGTCCAGGGG	R	Cloning
ORF63 (BamHI)	TACCGAGCTCGGATCCATGGACGGCACAGACGCT	F	Cloning
ORF63 (NotI)	CTCCCTCGAGCGGCCGCCCGATTTCGACAAACAGTTTCCG	R	Cloning
ORF64 (EcoRI)	AGTGTGGTGGAAATTCATGGCAGCCCAGCCTCT	F	Cloning
ORF64 (NotI)	CTCCCTCGAGCGGCCGCCCTCCCAAGTACCACTTCTTTATTCTGTCA	R	Cloning
ORF65 (KpnI)	GCTTGGTACCATGTCCAACCTTAAGGTGAGAGACCC	F	Cloning
ORF65 (XhoI)	CTCCCTCGAGCGATTTCTTTTTGCCAGAGGGGG	R	Cloning
ORF66 (EcoRI)	GGTGGAATTCATGGCCCTGGATCAGCG	F	Cloning
ORF66 (NotI)	TCGAGCGGCCGCCCTCCGGAGGAACACTTCCCG	R	Cloning
ORF67 (EcoRI)	GGTGGAATTCATGAGTGTGTTGGTAAGCGTGTAG	F	Cloning
ORF67 (NotI)	TCGAGCGGCCGCCCTCCGCTGGGCCTCATCC	R	Cloning
ORF67.5 (EcoRI)	GGTGGAATTCATGGAGTACGCGTCTGACCAG	F	Cloning
ORF67.5 (NotI)	TCGAGCGGCCGCCCTCCGGGCCGTGCC	R	Cloning
ORF68 (BamHI)	GCTCGGATCCATGTACAGGCAGAAGCTGG	F	Cloning
ORF68 (XhoI)	CTCCCTCGAGTCCAGCGTACAAGTGTGACGTC	R	Cloning
ORF69 (EcoRI)	GGTGGAATTCATGGAGACCCCGATATGCACC	F	Cloning
ORF69 (NotI)	TCGAGCGGCCGCCCTGATAGGGCGTTGACAAGTGC	R	Cloning
ORF70 (EcoRI)	GGTGGAATTCATGTTTCCGTTTGTACCTTTAAGCTTGT	F	Cloning
ORF70 (NotI)	TCGAGCGGCCGCCCGATACTGCCATTTCCATACGAATGG	R	Cloning
ORF74 (vGPCR) (NotI)	GAAGGGGGCGGCCGCAATGGCGGCCGAGGATTC	F	Cloning

ORF74 (vGPCR) (NotI)	TAGACTCGAGCGGCCGCTACGTGGTGGCGCCG	R	Cloning
ORF75 (FGARAT) (BamHI)	TACCGAGCTCGGATCCATGTTTGTGCTAGCTTTTATACAAGCCG TACG	F	Cloning
ORF75 (FGARAT) (NotI)	CTCCCTCGAGCGGCCGCTGAGTGGTGGTCGTTGATCTTCT	R	Cloning
K8.1 WT	CCGGCAGCAATATAAAAAGGGACC	F	EMSA
K8.1 WT	GGTCCCTTTTATATTGCTGCCGG	R	EMSA
K8.1 TATT:CCC C	CCGGCAGCAACCCCAAAGGGACC	F	EMSA
K8.1 TATT:CCC C	GGTCCCTTTGGGGTTGCTGCCGG	R	EMSA
ORF57 WT	TTAATCCCACTATATAACCTGGCT	F	EMSA
ORF57 WT	AGCCAGGTTATATAGTGGGATTAA	R	EMSA
ORF24 stop	CCGAGCGCCTCCCTGACGACGAGTCCGCAGACCACGTGTTAAC AAGCGACTTGGGAATCAAGGATGACGACGATAAGTAGGG	F	BAC mutagenesis
ORF24 stop	CAGCAGAATATTTCCAGCTGTGATTCCCAAGTCGCTTGTTAA CACGTGGTCTGCGGACTCAACCAATTAACCAATTCTGATTAG	R	BAC mutagenesis
ORF24 N FLAG	GGCGAGGTACGGGAAAAGGTCGTTGCTCCAAGGTCGCCTCCAT GGACTACAAAGACGATGACGACAAGATGGCAGCGCTCGAGGG CCCAGGATGACGACGATAAGTAGGG	F	BAC mutagenesis
ORF24 N FLAG	CGCTCGGTGGCAGTAGTAGGGGGCCCTCGAGCGCTGCCATCT TGTCGTATCGTCTTTGTAGTCCATGGAGGCGACCTTGGAGC AACAACCAATTAACCAATTCTGATTAG	R	BAC mutagenesis
K8.1 Pr	GGGAGAACCATGCCAGACTTTG	F	qPCR
K8.1 Pr	GCATAGGATTAGGAGCGCCAC	R	qPCR
ORF52 ORF	AAATCGAAGCCAGGGTCAGG	F	qPCR
ORF52 ORF	CTCCTCTTCGTCGCCTGTTATTG	R	qPCR
ORF57 Pr	ATTAGGGTGAGCGAAGTCACG	F	qPCR
ORF57 Pr	GCTTGTACCATGTCCTTTGGTT	R	qPCR
ORF57 ORF	GGTGTGTCTGACGCCGTAAAG	F	qPCR
ORF57 ORF	CCTGTCCGTAAACACCTCCG	R	qPCR
ORF38 ORF	TATCTGCAAACGTCCCTCAC	F	qPCR
ORF38 ORF	CATCCCTCTTCCCTCCATCCC	R	qPCR, Northern blot
K8.1 ORF	CCGTCGGTGTGTAGGGATAAAG	F	qPCR
K8.1 ORF	GTCGTTGTAGTGGTGGCAGAAA	R	qPCR
GAPDH Pr	TACTAGCGGTTTTACGGGCG	F	qPCR
GAPDH Pr	TCGAACAGGAGGAGCAGAGAGCGA	R	qPCR
ORF24 N- terminus	GCTCGGATCCATGGCAGCGCTCGAGGG	F	Cloning

(BamHI)			
ORF24 N-terminus (NotI)	TCGAGCGGCCGCTTGCTGCCAGAGTCCGCG	R	Cloning
ORF24 central domain (BamHI)	GCTCGGATCCATGTCTACAGTGTCAGACATGCTGGAAC	F	Cloning
ORF24 central domain (NotI)	TCGAGCGGCCGCTTGTTTCTGTGCTCAAGTAGGGAGAATATTCT	R	Cloning
ORF24 C-terminus (BamHI)	GCTCGGATCCATGCGTTCCCAAATACAGACGCTACACA	F	Cloning
ORF24 C-terminus (NotI)	TCGAGCGGCCGCAAGACCAGCGGACGGACG	R	Cloning
N425A, N427A ORF24	AGCTTATCTTAGTGTTAATCACCATGGCGGTGGCGAATATGGTGATCTTGAAGTTTTCCAAACTG	F	Site-directed mutagenesis
N425A, N427A ORF24	CAGTTTGGAAAACCTCAAGATCACCATATTCGCCACCGCCATGGTGATTAACACTAAGATAAGCT	R	Site-directed mutagenesis
N518A, N520A ORF24	TCCGTGCAGGTCCAACGTGGAGGCCCAAGCTGCTATCGATACAGGAAATATG	F	Site-directed mutagenesis
N518A, N520A ORF24	CATATTTCTGTATCGATAGCAGCTTGGGCCTCCACGTTGGACCTGCACGGA	R	Site-directed mutagenesis
5aa ORF24	CCTTTTTCGCCTGCAGAGCGATACGCGCAGCAGCCGCGGCGG AACGCCTCCACCCTTTTATACATC	F	Site-directed mutagenesis
5aa ORF24	GATGTATAAAAGGGTGGAGGCGTTCCGCCGCGGCTGCTGCGCGTATCGCTCTGCAGGCGAAAAAGG	R	Site-directed mutagenesis
3aa ORF24	TTTTTTCGCCTGCAGAGCGATACGCAGAGCAGCCGCGGGGAA CGCCTCC	F	Site-directed mutagenesis
3aa ORF24	GGAGGCGTTCCCCCGGGCTGCTCTGCGTATCGCTCTGCAGGC GAAAAA	R	Site-directed mutagenesis
UL87 (EcoRI)	AAAAGAATTCATGGCCGGCGCTGC	F	Cloning
UL87 (XhoI)	AAAACCTCGAGTCGTGATGCAAACCGCAC	R	Cloning

BcRF1 (EcoRI)	GGTGAATTCATGACACAAGGTAAGAGGGAGATGG	F	Cloning
BcRF1 (NotI)	TCGAGCGGCCGCGCCACTTGAGCATCACGGCAGTG	R	Cloning
mORF24 (BamHI)	TACCGAGCTCGGATCCATGACAATATTCTTACCAGTATTCTG TGATTTGC	F	Cloning
mORF24 (NotI)	CTCCCTCGAGCGGCCCGCCGGAGTCTGGTTGGCAAGG	R	Cloning
ORF8	CCTTCTTGGTGGTACTTGTCTTTGG	R	Northern probe
ORF65	GTTATACTGCTTCCCGAGACCC	R	Northern probe
ORF25	GAGGTGTGCCTTGAACATGGATA	R	Northern probe
K8.1	GTCGTTGTAGTGGTGGCAGAAA	R	Northern probe
7SL	GCTCCGTTTCCGACCTGGGCC	R	Northern probe

Strep K1, strep K12 (Kaposin A, Kaposin B, Kaposin C), and strep ORF72 (vCyclin) were kind gifts from D. Ganem. Strep ORF73 (LANA) was a kind gift from L. Coscoy.

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